KERR

The Bacillus Welchii (Bacillus Aerogenes Capsulatus) Isolated from the Human Intestine

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THE BACILLUS WELCHII (BACILLUS AEROGENES CAPSULATUS) ISOLATED FROM THE HUMAN INTESTINE

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Josephine Ellrod Kerr

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THE BACILLUS WELCHII (BACILLUS AËROGENES CAPSULATUS) AS ISOLATED FROM THE HUMAN INTESTINE

A. Historical review of the literature.

The bacillus is described under a number of names. Welch, in America, named it Bacillus aërogenes capsulatus; Fränkel,[7] in Germany, named it Bacillus phlegmones emphysematosae. Welch[36] and Fränkel agree that the organisms described by them are identical. Veillon and Zuber,[38] in France, named it Bacillus perfringens; Achalme,[2] in France, found it in cases of acute articular rheumatism and named it Bacillus of rheumatisme articulaire. Welch[13] states that after studying a culture of E. Klein's Bacillus enteritidis sporogenes he found it to be identical with his Bacillus aërogenes capsulatus. Kamen[17] considers the Granulobacillus saccharobutyricus immobilis liquefaciens of Schattenfroh and Grassberger[11] to be identical with Fränkel's"gas bacillus". Kemp[18] also considers it to be identical, probably, with the Clostridium butyricum of Prazmowsky. It is commonly called the "gas bacillus".

Metchnikoff[25] states that the bacillus was first described by Rosenbach, in 1884, in his "Monographie des microbes des maladies infectieuses des plaies", and later observed by Doyen, Levy and Klebs, and that Achalme, in 1891, obtained the first cultures. In 1889, Fränkel[6] described "rod-like bacteria, which morphologically have a great similarity to the anthrax bacillus" in stained sections of stomach from a case of gastritis acuta emphysematosa. He did not cultivate it. Welch and Nuttall[34]

published their first observations in 1892. They showed the relation of the bacillus to foamy organs and to the presence of gas in the blood-vessels at autopsy and in other parts of the body in which it is not ordinarily found. Fränkel,[7] in 1893, showed the relation of the bacillus to gas phlegmons. Since these first descriptions much work has been done upon the morphology, cultural properties and pathology of the bacillus, or group of bacilli, and the results show, as Passini[28] has pointed out "that this bacillus belongs to a group of anaërobic bacteria, which, by their activities form butyric acid from sugars, and that its morphology and bio-chemistry are far more complicated than its discoverer had thought".

As regards the morphology, both Welch and Fränkel first described the bacillus as asporogenic. Later Fränkel observed spores in cultures containing sodium formate and Dunham,[5] in 1897, observed spores in cultures upon blood serum. Achalme[1] and others have been able to bring about sporulation by incubating in sealed pipettes the subcutaneous exudate of guinea pigs or rabbits or the amniotic fluid of a female animal, infected with the bacillus. Grassberger[11] in 1900 was the first to cultivate spore-bearing rods and clostridium forms, and he showed that abundant spore-formation may be brought about by continual transplantation of the spores upon a suitable medium. He used alkaline starch-agar, containing 1 gram of starch to 1 liter of agar. The alkalinity plays an important part. Grassberger and Schattenfroh[11] found that if the bacilli produce an acid reaction in the medium that spore-formation does not take place and vegetative cells, only, are found. Passini[28] also was able to get spore-bearing rods by

transplantation of the spores upon solidified blood serum or upon egg-white cooked at 145° C and immersed in bouillon. Passini, working with a series of strains of Fränkel's "gas-bacillus" has shown that by suitable cultivation an asporogenic, non-motile bacillus may be changed into a sporogenic, motile one. Repeated transplantations of spore-material finally brought about cultures in which nearly every rod forms a spore. Grassberger[12] observed the same morphological change in the case of the Bacillus of symptomatic anthrax. Passini[28] observed that the change in morphology is accompanied by a change in bio-chemistry, that is, the non-motile asporogenic micro-organism cultivated upon sugar-containing media causes, almost exclusively, fermentation. This same strain cultivated in its sporulating form upon sugar-poor, protein-containing media, breaks up the protein, energetically, with the formation of putrefactive products.

Many investigators agree that the bacillus is present in the intestine of man in spore-form, and that it may be isolated from the feces in cultures from pasteurized spore-material.

Two types of involution forms are mentioned. Kamen[16] considers the involution forms developing in glucose gelatine stab cultures to be "rudimentary" spore forms. He says that the form is too definite and the staining properties too well retained to consider them involution forms. However, his preparations did not take the specific spore-stain and the cultures show no greater resistance to heat than spore-free agar cultures. Welch,[34] also, mentions the appearance of involution forms upon this medium. He calls them simply involution forms and attaches no other significance to them. Welch[34] mentions small coccus-like forms in old

agar cultures.

Grassberger and Schattenfroh[11] and Kemp[18] consider the formation of granulose as a sign of involution or degeneration change within the cell. Grassberger[12] has found in cultures of Granulobacillus saccharobutyricus immobilis liquefaciens, of Granulobacillus saccharobutyricus mobilis and of the Bacillus of symptomatic anthrax, rods which stain wholly or partly black-violet with Lugol's solution. Grassberger[12] says this reaction is characterized by the diffuse appearance of a substance within the cell stainable with iodine, which he calls granulose. It is a substance related to starch and is usually formed in starch - contain-media. Nothnagel[27] in 1884, first observed that there occur, in human feces, many microörganisms which are characterized by staining dark blue with iodine solution. Many of these have a form similar to that of yeast cells, but are distinguished from the latter, which always stain yellow to yellow-brown with iodine, while these granulose bacteria stain blue to blue-black. Kemp[18] observed that the appearance of large numbers of granulose microbes is always a sign of pathological composition of the stools. P. Selter, according to Kemp, concludes (in the case of nurslings) that granulose bacteria in the feces are a sign of poor digestion of carbohydrates. Strasburger[30] observed them especially in intestinal fermentation dyspepsia of nurslings in the stools characterized by abundance of carbohydrate and gas. From a large number of examinations of stools Schmidt[30] found that the appearance of granulose bacteria is a direct sign of poor starch digestion. Strasburger[30] noticed that such stools always have an odor of butyric acid. Meyer, Kemp states, found granulose bacteria in

6 out of 17 cases of fermentation dyspepsia. Passini cultivated aërobic butyric acid bacilli from the feces and demonstrated their granulose reaction in pure cultures. The fecal bacteria which contain granulose appear as rods, or spindles. Grassberger has shown that bacilli and spores containing granulose are hard to cultivate and for this reason regards them as involution forms. Kemp[18] decided that this may account for his obtaining positive results in cultures in only 3 of the 17 cases of "fermenting stools" which he studied. He concluded that as long as the bacteria grow energetically they digest the carbohydrate and do not deposit granulose, that this happens when their vitality wanes. Grassberger[12] has shown that granulose formation has nothing to do with typical spore-formation and that spores which have the greatest vitality are free from granulose.

Many conflicting descriptions have been given of the behavior of the bacillus in gelatine culture. In Welch's[34] first description of the bacillus he classed it among the non-liquifiers of gelatine. Later he[35] states that the bacillus liquifies gelatine slowly, and that this property varies with the strain, some strains liquifying the medium rapidly while others do so scarcely at all. Kamen[16] says that the liquifaction of gelatine is inconstant and depends upon different factors, and is not only dependent upon the peculiarity of the different strains, but the composition and consistency of the gelatine, and the circumstances which influence the condition of the latter, such as, mode of sterilization and the temperature of incubation of the cultures. In this way he accounts for the different results of different authors.

Achalme[2] was able to cultivate a number of anaërobes, among

them, Bacillus perfringens, upon a medium consisting of 2 grams of coagulated egg-white and 10 c.c. of tap water. The medium is sterilized for 15 minutes at 120° C. He obtained good growth with or without the addition of carbohydrate. He found that if the sugar is not attacked then the egg dissolves, and spores appear in abundance, if the carbohydrate is split then the egg remains intact. Bacillus perfringens was found to attack carbohydrate if it is present, with the formation of gas, but if no carbohydrate is present the egg is digested and spores are formed. He found that the following are always associated: (1) fermentation of carbohydrates, acidity, intact albumen, absence of sporulation or, (2) carbohydrates not fermented, alkalinity, solution of albumen, sporulation. He obtained the same results with fibrin used in the place of egg albumen.

The fermentative activity of the bacillus is well recognized. It ferments carbohydrates with special energy, the di - and monosaccharides with the formation of acids and gas. Welch[36] observed the largest gas production in the media containing lactose and least in media containing saccharose. Grassberger and Schattenfroh[11] give complete chemical analyses of the products of growth of Granulobacillus saccharo-butyricus immobilis liquefaciens from different sources upon different media, the principal products being butyric acid, lactic acid, carbonic acid, hydrogen and sometimes alcohols in small quantities.

Welch's[34] incubated rabbit test is a characteristic test for the bacilli of this group and this test shows the power of the bacillus to produce gas from protein material.

That the bacilli of this group are pathogenic there is little doubt, and as with other pathogenic bacteria the virulence varies with the source of the culture, conditions and length of time of cultivation upon artificial media, and other factors. The greatest number of human infections reported have been extensive, lacerated wounds in which dirt was ground into the tissue, the bacillus being present in the soil in spore form. In these cases the infection is in the form of emphysematous gangrene. A number of cases of endometritis following abortions have been reported, the infection being due to this bacillus; in these cases it sets up a fatal infection with invasion of the bacillus into the blood. Some cases have been reported of invasion from the intestine in case of a perforation and development of the bacillus in the tissues causing death. All authors agree that virulent infections are characterized by rapid liquefaction necrosis of tissue accompanied by gas production. Fränkel[7,8,9] and Hitschmann and Lindenthal[14,15] arrived at different conclusions as to the pathological process in the case of gas phlegmons. Fränkel considered it to be an inflammatory process accompanied by infiltration with leucocytes and Hitschmann and Lindenthal considered it to be a "fermentation necrosis" with little reaction on the part of the body cells. Kamen[17], after a careful study of the histological sections from guinea pigs in which gas phlegmons had been produced, concluded that the anatomical changes may be either necrotic or inflammatory, or that both types may be present at the same time. He concluded that the action of the "gas bacillus" in guinea pigs is analogous to that in man. His investigations show that the action of the "gas bacillus" is a local one and that in-

vasion of the bacilli into the blood and internal organs is usually post-mortem. In autopsies made immediately after death there are found in the heart blood, liver, spleen, etc. none or only a few isolated rods, while in animals incubated after death there is abundant development of the rods in the internal organs and tissues.

E. Klein[20] seems to be the only investigator who has found the bacillus to be capable of setting up a pathological condition in the intestine. Since he was among the first to isolate the bacillus from the intestine, and since his work upon the bacillus as isolated from the intestine is in a measure pioneer work, a more detailed discussion of his investigations may not be out of place. In 1895 Klein[20] isolated, from the stools of patients suffering from an epidemic of diarrhoea in St. Bartholomew's Hospital in London, an anaërobic spore-bearing bacillus, which he named Bacillus enteritidis sporogenes, and which he characterized as an anaërobic, pathogenic, intestinal bacillus. Klein suspected that the poisoning was due to the milk which had been drunk by the patients and the nurses succumbing to the diarrhoea. He procured samples of the milk and found his bacillus to be present in the milk which was furnished to the hospital. After a careful study of Klein's description of his method of procedure and his results it seems highly probable that in some cases at least he was working with mixed cultures, as he himself, in fact later admits.[23] In isolating the bacillus from the intestine he took a mucous flake from a stool and inoculated it into a tube of liquid gelatine, heated the tube at 80° C for 15 minutes, solidified the tube and incubated it at 20° C, or he inoculated the mucous flake into

sterile milk, and according to the procedure of Botkin, heated it in the flasks at 80° C for 15 minutes and incubated the flask at 37° C. Then from the whey of the milk or the gelatine culture he made inoculations directly into animals and described the pathological changes as due to one bacillus, his Bacillus enteritidis sporogenes - no reference is made to plating the culture before the inoculation. It is probable that there were also present spores of other anaërobes in the culture inoculated, because subsequent investigations have shown that there are several species of spore-bearing anaërobes present in the human intestine. His description of the flagella-stained preparations of his bacillus is as follows: "Long rods possess at one end 1, 2, or 3 flagella, the short rods have 6 to 8 flagella on one end and 2 to 3 on the other, always attached laterally to the rounded ends. One finds in the preparation numerous free flagella, or bundles and tufts" . . . . "The flagella are twisted, wavy, or spirals; many are of striking length. In many preparations is the number of the torn-off, spiral flagella so great, that it appears, as if we had to do with a culture of fine, lightly-stained spirilla". In preparations which show so many broken off flagella it would seem that one could hardly determine whether they are arranged upon the bacilli peritrichiately or otherwise. He[21] described two distinctly different actions of the bacillus, both in milk and in gelatine. He says "when the bacillus, in spore-form is planted into sugar-gelatine, the typical rapidly liquefying colonies are formed, but when the vegetative cells are transplanted into sugar gelatine, the resulting colonies, at least for many days are not liquefied." With regard to milk he says, "Spores which have been

transplanted through a series of sugar gelatine tubes do not longer produce coagulation and rapid and abundant gas-formation with acid reaction in the medium and butyric acid odor, but gas formation is slower, after a week the milk is changed into a white, coagulated mass or yellowish-colored whey, with alkaline reaction and foul odor." Also he says in case of milk cultures with acid reaction which he designates as "typical", "the rods are present as short, sporeless rods" and in the other type of milk culture which he designates as "atypical", "they grow as threads". "Inoculation in sugar-gelatine of a "typical" milk culture produces quickly liquifying colonies composed of bacilli which form spores readily. The "typical" milk culture is virulent for guinea pigs, the "atypical" one causes even in larger doses only a gelatinous swelling, not death." In his description of the bacillus which he called Bacillus cadaveris sporogenes in 1899, he corrects his statement made in previous publications that Bacillus enteritidis sporogenes cultivated from the subcutaneous gangrenous tissue of guinea pigs gradually by further transplantation gave "atypical" milk cultures and lost its virulence. He concludes from further study that these "atypical" cultures are not really Bacillus enteritidis, but are due to a secondary invasion of Bacillus cadaveris[23] from the intestine into the gangrenous subcutaneous tissue. It would seem all the more probable that, in those cases where he made the inoculations into guinea pigs directly from the whey of milk cultures from pasteurized feces or other source, the spores of his Bacillus cadaveris (Bienstock[4] found it to be identical with his Bacillus putrificus) were already present in the inoculated material. Harris, in his edition of Muir and Ritchie[26]

states that "not a few American and Continental workers exhibit some hesitancy in accepting the status of Bacillus enteritidis sporogenes as established by Klein; for his published descriptions are not free from the suspicion of the existence of cultural impurities involved in the technic employed." Welch[13] obtained a pure culture of Bacillus aërogenes capsulatus from a culture sent to him by Klein. Metchnikoff[25] states that the bacillus found by him in nearly every stool examined, and which he calls Bacillus sporogenes, corresponds most closely to the Bacillus enteritidis sporogenes of Klein. We have found this bacillus (which is described by Metchnikoff) in many of the stools examined. This bacillus is distinctly different from the "gas bacillus" in nearly all of its properties. Later investigations have shown, for example those of Korentchevsky[24] upon Bacillus perfringens and Bacillus putrificus, that the intestinal anaërobes, when fed to young dogs do not produce severe effects. Glynn[10] has shown that large numbers of the bacilli (Bacillus aërogenes capsulatus) may be swallowed by a healthy man without producing any immediate ill effect.

An important point brought out by Klein's observations is that in cases of severe intestinal irritation large numbers of spores of anaërobes are excreted. Whether these anaërobic bacteria are taken in with food and set up this pathological condition by multiplying and producing poisonous substances in the intestine, or whether the poisons were already present in the food when it was taken into the digestive tract and that there they irritate the intestine and cause the multiplication of the anaërobic bacteria already present allowing them to be excreted in larger numbers than they ordinarily are, is a question. The increased fluidity

of the stools in these cases of poisoning undoubtedly furnishes a better medium for the multiplication of all intestinal bacteria. Another hypothesis is that the bacteria normally present may under abnormal conditions become virulent. It must be remembered in accounting for the etiological factor in any intestinal disturbance that the normal flora is made up, not of one, but of many species of bacteria and that the problem becomes a complicated one for this reason. It is improper to pick out one of these species, although it be the predominant one in the stools, and say that it is the cause of the trouble except in those cases where the specificity can be absolutely proven.

The characteristics of the disease phenomena have led most investigators to believe that the Bacillus aërogenes capsulatus forms poisons. Kamen[17] was the first to demonstrate a poison. With an 8-day glucose bouillon culture which had been filtered through a Pasteur-Chamberland filter he was able to demonstrate a haemolytic action upon blood. His culture was obtained from a guinea pig dead with gas phlegmon. He says that this haemolysin is present in varying amounts and is destroyed by long incubation. Herter[13] was able to confirm Kamen's results. He found that in a 5-day culture of Bacillus aërogenes capsulatus in blood bouillon, haemolytic substances are produced. One-half to 1 c.c. of the culture filtrate produced hemolysis of rabbit's blood cells. Herter[13] considers this haemolytic action to be due, in part, to the formation of ammonium butyrate, and in part to some unknown substance. Herter[13] has emphasized this haemolytic action of the bacillus and considers it of importance in connection with pernicious anaemia. Kamen[17] was also able to demonstrate a toxin

which attacks the leucotytes. He says that both of these substances are present in relatively small amounts in cultures and concludes that, in cultures, the "gas bacillus" forms only small amounts of relatively feeble toxins. Passini[28] working with sporogenic strains of the bacillus isolated from the intestine demonstrated two poisons. One, a very virulent poison, is produced in cultures prepared in the following manner: Bouillon is made from beef which has been digested for about 3 days with trypsin ferment. To this bouillon 2% glucose is added and the medium is sterilized. The cultures are allowed to grow 2 to 4 weeks in a hydrogen atmosphere. One-half to 1.5 c.c. of the culture filtrate injected intravenously into rabbits kills in ½ to 1 minute. The poison affects the respiratory centers. The other poison, less virulent, is produced in 2 to 4 weeks-old glucose bouillon cultures containing calcium carbonate. Cultures incubated upon this medium for this time are alkaline in reaction. In guinea pigs injected subcutaneously with 3 c.c. of a culture filtrate there develops in 15 to 20 minutes a hemorrhagic oedema and the skin over the abdomen shows small hemorrhagic spots. The oedema spreads quickly over the anterior abdominal wall, and the central part becomes necrotic. The disease spreads locally without becoming generalized and ends within a few days with the formation of a small necrotic area. Passini showed that these toxic effects are not due to the presence of butyric acid in these cultures because the effect is produced as well in alkaline as in acid reaction. He concludes that these poisons are not bacterial toxins, because they are not destroyed by heat and are dialyzable, but that they probably belong to the ptomaines. Herter[13] has been unable to confirm Passini's results with the asporogenic form of

the bacillus. Korentchevsky[24] is at work upon the toxicity of Bacillus perfringens and its toxins when introduced into animals by the mouth and by the rectum. The bacteria are isolated from the species of animal used in the experiment. He has found that of the most active toxin, 1 to 3 c.c. per kilo body weight, injected intravenously into a grown rabbit kills in 3 to 4 hours. He fed young dogs with ½ liter of culture of Bacillus perfringens in meat bouillon. He found that the only effects were a certain arrest in the weight increases and that the feces were somewhat more fluid and putrid. He tested for agglutinins, precipitin and fixateur. He found fixateur constantly present. The bacteria fed became predominant in the feces of the dogs.

In their attempts to prove the close relationship or identity of the cultures of the non-motile butyric acid-forming bacilli, some investigators have turned to the agglutination tests as the safest criterion for judging. Kamen[17] obtained negative results with rabbits immunized with cultures of this bacillus injected subcutaneously. Werner[37] worked with 10 strains of butyric acid bacilli. They are as follows:

1. Strain GB from the subcutaneous tissue of a man dead of gas phlegmon.

Strain GBN from kidney of the same case.

2. Strain T from a case which was suspected to be tetanus infection but proved to be infection with "gas bacillus".

3. Strain L from the "foamy" liver of a rabbit incubated 20 hours.

4. Strain G(ranulo) B(acillus) I from market milk.

5. Strain G(ranulo B(acillus) II obtained from L. Kamen.

6. Strain Fr. obtained from E. Fränkel, "gas bacillus".

7. Strain M from a gas-containing abscess at the site of inoculation of a guinea pig inoculated with anthrax.

8. Strain MKI from the subcutaneous tissue of a guinea pig inoculated from a rabbit with gas phlegmon.

9. Strain MKII. Same from another guinea pig.

10. Strain GPh from the bloody fluid from an arm fracture.

Werner cultivated all these strains alike upon sugar agar for some months. The procedure which he used for immunization is as follows: 20-24 hour old sugar agar plate cultures were suspended in salt solution. The culture was then heated for 1 hour at 60° C upon a water-bath and injected into the ear vein of a rabbit. Later, living cultures were injected, and the doses repeated and increased. The rabbits usually received 3, 5, or 6 injections in periods of 10 to 20 days. After the last injection the blood was drawn from the carotid artery and the serum collected. For the agglutination experiments the serum was diluted further with physiological salt solution in different amounts. One c.c. was then put into clean tubes and a 20 hour old bacterial culture was suspended and added. The tube was incubated 2 hours and studied microscopically and microscopically in hanging drop.

The following table shows the results which he obtained by this procedure.

	I	II	III	V
Strains	Immune sera of Strains			
	1. GBN	4. GrBI	3. L	6 Fr.
1. GBN	1:250 =+	1:10= -	1:10 = -	1:10 = -
3. L	1:10 = -	1:10 = -	1:200 = +	1:10 = -
4. GrBI	1:10 = -	1:1000 =+	1:10 = -	1:10 = -
5. GrBII	1:10 = -	1:600 = +	1:10 = -	1:10 = -
6. Fr.	1:10 = -	1:10 = -	1:10 = -	1:500 = +
7. M	1:10 = -	1:10 = -	1:10 = -	1:10 = -
8. MKI	1:10 = -	1:10 = -	1:10 = -	1:10 = -
9. MK II	1:10 = -	1:10 = -	1:10 = -	1:10 = -
10. GPH	1:10 = -	1:10 = -	1:10 = -	1:10 = -
Malig. Oedema	1:10 = -	1:10 = -	1:10 = -	1:10 = -
Symp.Anthrax	1:10 = -	1:10 = -	1:10 = -	1:10 = -

The table shows that positive results were obtained with the homologous strain only, in every case except the two strains of the granulobacillus and he considers these to be the same strain, probably. Werner finds two explanations for this behavior of otherwise closely related strains, either (1) that the gas phlegmon bacilli like the "coli species" lack the property of producing a typical agglutinin for their entire species, while one is produced for single strains or strains of like origin; or (2) that the large group of our so-called gas phlegmon bacilli may be divided into numerous species.

Passini,[28] on the other hand was able to obtain sera which would not only agglutinate the homologous strain but other strains, which showed, in culture, varying characteristics. Passini used old heated cultures from various media (sugar bouillon, gelatine, milk, egg-white and blood serum) and his results were positive in dilutions of 1:30 and 1:80. Werner makes the very fair criticism that Passini's technic is not beyond question. He says that by the use of old cultures and low dilutions confusing results are likely to be obtained.

That there has been considerable confusion with respect to the exact properties of the "gas bacilli" is apparent from the fact that they have been described in four different countries under six different names. Investigators who have studied the bacillus subsequent to these discoverers have taken their choice of names according as they were German, or French, or English, or American. A good deal of this diversity in the nomenclature is due to the circumstance that the bacilli show varying properties, dependent upon the source from which they are isolated, and the medium upon

which they are cultivated and that the pathogenic properties are very complex. Passini[28] and Grassberger[11] have been able to transform asporogenic strains into sporogenic ones by cultivation upon suitable media, this property of spore-formation which is ordinarily found, in the case of other species, to be a fairly constant characteristic, in the case of this species, is found to vary considerably under different experimental conditions. We have been able to confirm Passini's[28] results with strains isolated from feces. From the results of Grassberger[12], Kemp[18] and others there is little doubt that the "gas bacilli" constitute one group of the granulose bacteria found in the feces. We have cultivated the different strains of the "gas bacillus" isolated from feces upon starch-containing media, potato and potato-agar and have found that some of the rods contain granules which stain blue with Lugol's solution.

An important property of the bacillus is its capsule-formation. Welch[34] and Kamen[17] mention this property. Welch demonstrated the capsules with his capsule stain and Kamen[17] gives a photograph of a preparation stained by Loeffler's flagella stain. We have demonstrated capsules upon rods from animal tissues, and from artificial media containing blood or sugar by staining with the Hiss capsule stain.

We have been unable to confirm Achalme's[2] results by cultivation of our strains of the bacillus upon egg-white medium sterilized at a temperature of 120° C for 15 minutes. Strains of the Bacillus of symptomatic anthrax and the Bacillus of malignant oedema cultivated as controls did not digest the medium when no carbohydrate was present. The growth of our strains upon this

medium was very feeble, even when 3% glucose was added to the medium, growth was not much more abundant. For this there are two possible explanations: (1) that our medium lacked some constituent which Achalme's contained, but which he failed to mention, or (2) that our strains of the "gas bacillus" and the Bacillus of symptomatic anthrax and the Bacillus of malignant oedema do not grow readily upon the medium, and do not possess the property of attacking this native protein readily, as did the strains employed by Achalme. Our strains do, however, digest an egg medium rather energetically which is prepared according to the procedure described by Passini, in which the egg is heated in the autoclave at 144° C and then immersed in a little bouillon. The growth was energetic upon this medium.

From a study of the investigations upon the pathogenic effects of the "gas bacilli" we believe that the toxic effects of the "gas bacilli" when inoculated into the animal body are due to a number of poisons some of which are undoubtedly split products of the culture medium upon which the bacillus grows, (Animal tissue protein broken up into poisonous groups.)

Werner[37] and Passini[28] have arrived at different results in their investigations upon the agglutination phenomena in strains of the "gas bacillus". Werner obtained positive results with the homologous strain only, in every case except the two strains of the granulobacillus employed and he concludes from this that the gas phlegmon bacilli lack the property of producing a typical agglutinin for their entire species, or that they are separate, distinct species. Passini,[28] on the other hand obtained positive results with other strains than the homologous strain.

Schattenfroh and Grassberger[11] described their Granulobacillus saccharobutyrious immobilis liquefaciens as a non-pathogenic bacillus, but Kamen[17] was able to produce typical gas phlegmons with strains of this bacillus which he isolated from milk and concluded that the two bacilli are identical.

Until there is some positive and sure criterion for judging, one can not classify these bacilli into a single and well-defined species, on the other hand, one can not make separate species for bacilli which have so many characteristics in common and no definite and constant differences. Therefore in speaking of the bacilli, in general, it would seem more nearly correct to designate them as "the gas bacilli" or "the non-motile butyric acid bacilli" thus using some term which implies a common characteristic for the bacilli of the entire group. When studying specific strains one will then determine by his own judgment from the source of the culture, its properties, etc. which member of the group he has at hand

It was the purpose of the following experiments to show that the strain to be described is identical with the Bacillus aërogenes capsulatus of Welch and to point out the most important properties of the bacillus as isolated from the normal human intestine.

B. Distribution and Occurrence.

From a review of the literature and from the results of our own experiments it seems safe to conclude that the normal habitat of this bacillus is the intestine of man and some animals. Welch,[36] Passini,[28] Herter,[13] Stokes,[32] and Metchnikoff[25] have worked with strains from the intestine of man. Herter[13] has demonstrated its presence in the intestine of a large number of ani-

mals, herbivora and carnivora. We believe that no previous investigator has demonstrated its presence in such high dilutions as we have done, that is, in dilutions of 1:1000 and 1:5000 we have isolated the bacillus in spore form from nearly every stool examined. Schattenfroh and Grassberger[11] isolated their Granulobacillus immobilis from 80% of samples of market milk examined. There is every reason to believe that the bacillus is a contamination from the feces of the cow, feces being an almost constant constituent of milk under ordinary conditions. That the bacillus is also widely distributed in the soil is to be judged from its frequent occurrence in infections where the soil is ground into the wound. It is also found in street dust, water and sewage, in fact every place which is contaminated with excreta. It seems fair to assume that the bacillus has adapted itself to a parasitic life in the intestine as has the colon bacillus, and that it is capable of developing there and forming its spores.

C. General Routine procedure for collecting the cultures.

Source of Material.- The species to be described were obtained from the feces of 12 individuals, the subjects of a metabolism experiment to determine the effect of cured meats upon human health; the work being carried on under the direction of Professor Grindley in the Laboratory of Physiological chemistry at the University of Illinois. A complete bacteriological examination was made upon a stool from each individual every 16 days. A detailed description of the source of material, preparation of the suspension for bacteriological investigation and direct quantitative results may be found in the Journal of Infectious Diseases, April 1, 1909.

It is sufficient to explain here that the feces are collected in a sterile basin, taken directly to the laboratory, and thoroughly mixed with sterile instruments. 0.5 grams are then weighed out accurately upon the analytical balance in a sterilized, accurately weighed, graduated 50 c.c. measuring flask. The material is then mixed with 50 c.c. of sterile physiological salt solution, which is added to the flask and thoroughly shaken and stirred until a very homogenous suspension is obtained. From this 1:100 suspension dilutions are made for all of the bacteriological work.

Methods for obtaining cultures of the spore-bearing anaërobes.- From this 1:100 suspension four higher dilutions are made by mixing in sterile test tubes 1 c.c. of each preceding dilution with 9 c.c. of physiological salt solution. Dilution 1:1000 is used for the anaërobic cultures. The suspension is heated upon a water-bath regulated at 80° C for 15 minutes and then promptly cooled.

a. Plate cultures.- From this heated suspension in which the spores alone are capable of development, two blood agar plates are made by measuring out accurately ¼ c.c. (plate I) and ½ c.c. (plate II) into sterilized Petri dishes. To each of these plates 10 to 12 drops of naturally sterile defibrinated dog's or rabbit's blood and liquid agar cooled to 45° C are added, thoroughly mixed by rotation, medium allowed to solidify and the plates placed in a Novy anaërobe jar. Hydrogen gas, generated by a Kipp generator, passes through the jar for about 2 to 3 hours, the last traces of oxygen are absorbed by pyrogallic acid and alkali, which are mixed by a siphon flask arrangement within the jar (see fig. I). Anaërobic conditions are tested by means of a tube of glucose gelatine to which a little methylene blue has been added. The jar is incu-

bated at 37° C for 48 hours. Subcultures are then made from the colonies. These subcultures consist in (1) a test-tube culture (plain agar streak and stab) grown aërobically or anaërobically as the culture requires; (2) sugar blood beef tea fermentation tube. From the test-tube culture, if growth is obtained, sub-cultures are made into sugar-blood fermentation tube or litmus milk fermentation tube or into the blood-vessels of an animal.

b. Fermentation tubes.- Three kinds of media are used for the fermentation tube culture of these anaërobes. (1) Professor Theobold Smith[31] has shown that anaërobes grow well and may be kept for a long time in the closed arm of a bouillon fermentation tube to which a bit of sterile tissue has been added. Instead of the tissue bit, sterile blood may be substituted for convenience. One-half c.c. of the heated material (1:1000 suspension) is inoculated and is well mixed with the medium in both arms of the tube; (2) a litmus milk fermentation tube is inoculated in the same manner as above. The medium is made by adding a few drops of sterile litmus solution to a sterile milk tube; (3) a sugar-free bouillon fermentation tube containing a few pieces of sterile coagulated egg-white is inoculated as above. The medium is prepared by adding a few pieces of cooked egg-white to a tube containing sugar-free bouillon and re-sterilizing the tube at 120° C for 15 minutes. The three tubes are incubated 24 hrs. at 37° C or longer, if necessary.

These methods were used as the routine procedure for collecting cultures of anaërobic bacteria from the spore-material from each sample. They have proven valuable also for a rough classification of the species found. It will be noted that the dilutions

used are high and that cultures were obtained repeatedly, hence the species obtained must have been present in the feces in sufficient number to exclude the likelihood of their being accidental "wilde keime", and to warrant the assumption that they are normal inhabitants. (It is evident since only spore-matierial was employed for these cultures that certain asporogenic species are excluded - that is, anaërobic cocci, and diplococci, and asporogenic bacilli).

Cultures of anäerobes were collected during a period extending from Feb. 20, 1908 to Aug. 31, 1908. During this time about 150 pure cultures were obtained from colonies which had developed upon the blood agar anaërobic plates and which had been transplanted upon plain agar and stored in the refrigerator. The fermentation tubes which had been inoculated with spore-material and incubated, and which usually contained mixed cultures were also preserved at ice-box temperature to aid in the determination of the species. These cultures were stored until October 1, 1908 when experiments were begun for the purpose of determining species. Owing to the press of work a culture could not be completely studied at the time it was isolated. Some of the cultures obtained were difficultly cultivable upon the media employed and died out and could not be recovered when the time came to study them.

All those cultures which showed similar characteristics were grouped into one group. From this group a single culture was selected, plated upon blood agar anaërobically and its morphology, cultural characteristics and its bio-chemical properties and pathogenicity studied.

It is the purpose of this paper to describe only one group,

the Bacillus aërogenes capsulatus.

D. Description of a strain of the bacillus isolated from normal adult feces.

a. Morphology.

The strain selected for detailed study of this bacillus for its identification is D265 (1). It was isolated from a stool of normal character. The pure culture was obtained from a thin, surface, haemolytic colony upon a blood agar plate inoculated with spore material which had been incubated anaërobically for two days at 37° C. This colony was composed of plump, non-motile rods and contained no spores.

As has been pointed out by Welch,[36] Passini[28] and others, the morphology of this bacillus varies somewhat with the medium upon which it is cultivated. The rods, as sketched with the camera lucida, are somewhat smaller in the case of smears from the tissues of an animal, than when cultivated in a sugar blood bouillon fermentation tube. This is not due to a variation in the thickness of the capsule but the rod itself varies in size. Also the capsule varies in thickness, in cultures containing no blood only thin capsules are developed. In glucose gelatine stab cultures cultivated aërobically at 60° F the rods are much longer and thinner than are ordinarily found, some are 10 to 15 microns long and only .5 to .75 microns in width, and they tend to grow in threads.

The strain employed for identification, as ordinarily found, is a plump, non-motile rod with rounded ends, growing singly or in pairs or in short threads of 3 or 4 rods. In cases where the rods occur in pairs the two adjoining ends are somewhat squared.

Table of Measurements

		In Hanging Drop from agar stab 24 hr.		In Gram-stained Preparation from agar stab 24 hr.		In capsule stained Preparation from sugar blood bouillon.	
		Length	Width	Length	Width	Length	Width
Cells	(Maximum	7.6*	1.7	6.6	1.7	6.6	2.5
	(Minimum	2.6	1.1	2.6	.7	2.8	1.9
	(Mean	4.6	1.3	4.3	1.3	4.7	2.1
Spores	(Maximum	2.5	1.2				
	(Minimum	.8	.8				
	(Mean	2.0	1.1				
Capsules	(Maximum						.72
	(Minimum						.36
	(Mean						.49

* Microns.

No flagella are observed upon bacilli stained with Loeffler's flagella stain. Young agar cultures of several strains of the group were incubated anaërobically for about 6 hours and smears stained show no flagella upon the bacilli.

In media containing blood and in the tissues of the animal body the rods show, when stained by the Hiss capsule stain, capsules averaging about .5 microns in width.

The rods stain well in aqueous solutions of the anilin dyes. They take Gram's stain, but irregularly; even in young cultures there may be found, in a microscopic field, about as many red, counter-stained rods as blue ones.

Our strains of the bacillus agree with the description of other investigators in that they do not form spores readily upon artificial media, although they were originally obtained from spore-material, as has been previously described. A sporogenic

form of the bacillus was obtained by the following procedure described by Passini.[28] Take a culture (in this instance a Loeffler's blood serum culture) in which a few isolated spores have been observed and heat it upon a water-bath at 80° C for 15 minutes. Inoculate this spore-material into tubes containing egg-white medium sterilized at 144° C and plain solidified ox blood serum or Loeffler's blood serum. In cultures incubated anaërobically at 37° C for 5 to 10 days spore-bearing bacilli and free spores were observed. The process repeated 5 times or more gave rise to cultures in which there is abundant spore-formation. For each transplant a few loopfuls of the culture, suspended in physiological salt solution, were heated at 80° C for 15 minutes and this spore-material inoculated. By means of this method it has been possible to get spore-containing cultures of D265 (1); F225, E257, G264, H204, upon plain solidified serum, Loeffler's serum, and egg-white medium. The egg-white medium is sterilized at 144° C for 1 hour. It has been possible to confirm Passini's observation that elimination of vegetative cells by pasteurization and cultivation upon egg-white medium, which had been heated at 144° C for 1 hour, produces cultures which though, at first, contain only a few isolated spores, by continued transplantation contain many spores and spore-bearing rods. Control cultures made at the same time from material containing vegetative cells alone show no spore formation upon the medium employed. A culture of D265 (1) upon Loeffler's serum incubated 5 days at 37° C anaërobically showed more spores than vegetative cells per microscopic field. This was the sixth transplant of spore-material upon this medium.

The spores are large oval and highly refractive, bulge the cell wall and occur at different positions within the rod. They are most frequently observed within clostridium forms. Spores of this strain (D265 (1)) of the bacillus have been observed in only one other instance, an agar stab culture from the tissue of a guinea pig dead after inoculation with the strain. Spores could not be obtained from cultures upon starch-agar or potato-agar.

Involution forms appear in glucose gelatine stab cultures incubated at 60° F. The rods are thinner, longer and tend to grow in chains. "Knobbed", bent, and irregularly-shaped forms are observed. The preparations from our gelatine stab cultures are similar to those photographed and described by Kamen.[17]

b. Cultural Features.

Plain agar stroke and stab culture incubated at 37° C, abundant white irregular growth develops along the line of inoculation and heavy growth in the liquid of condensation, with the formation of many gas bubbles which riddle the medium. No growth develops on the surface of the slant if the culture is incubated aërobically. It has been found convenient and often times time-saving to make both a stroke and a stab inoculation upon the same tube of medium. If there is aërobic contamination it may readily be detected in such cultures.

Blood agar plate cultures show characteristic colonies. The cultures are made by dropping 10 to 12 drops of naturally sterile, defibrinated blood into a sterile petri dish, inoculating this blood with the culture and then pouring in the liquid agar cooled to 45° C and mixing by rotation. In some cases it is convenient in testing the purity of a culture to first allow the medium to

solidify in the plate and then make stroke inoculations upon the surface of the solidified medium. After 2 or 3 days incubation anaërobically at 37° C, thin, white, soft slightly spreading colonies develop upon the surface of the medium, or deep irregular, oval or "feathery" colonies develop in the deeper layers. In nearly every case these colonies are surrounded by a perfectly clear area, which is caused by a solution of the haemoglobin by the haemolysin produced by the bacteria. These haemolytic areas stand out as small, clear, circular spots.

In potato slant cultures inoculated upon the surface of the medium and also stabbed into it for a short distance and incubated anaërobically for 10 days at 37° C invisible growth developes. A few gas bubbles are formed in the liquid of condensation. The growth is composed of the plump, non-motile rods, most of which contain granules. Some of these granules stain blue with Lugol's solution. Some of the strains of this group, for example E257 (1) and G264, produce, when cultivated upon this medium, elevated, soft, circular, creamy-yellow colonies. These also consist of rods which contain granules staining blue with Lugol's Solution.

Loeffler's blood serum cultures incubated anaërobically for 9 days at 37° C contain abundant growth of the rods, some are slightly spindle-shaped, and tend to grow in threads. The medium is almost completely digested into a brown liquid which contains a few small gas bubbles and has a putrefactive odor. Upon this medium the rods grow very large and plump. Some free spores are observed in the culture. This strain of Bacillus aërogenes capsulatus liquefies the serum more slowly than strains of the Bacillus of symptomatic anthrax and the Bacillus of malignant oedema incubated

as controls. The latter liquefy the medium completely in 3 to 5 days.

In glucose gelatine stab cultures, incubated aërobically at 60° F, a white irregular growth appears along the line of inoculation within 5 days. There are gas bubbles through the medium and very slight liquefaction. At 9 days the culture is slightly liquefied along the line of inoculation. The medium is not completely liquefied after 3 months incubation at this temperature. The rods appear longer and somewhat more slender than are ordinarily found. Some "capitate" forms are seen and some involution forms. The rods tend to grow in short threads, some of the rods grow singly and they are of ordinary size. Plain gelatine cultures are not liquefied more rapidly than those to which glucose has been added. Also virulent cultures from animals dead in 24 hours after inoculation do not grow more rapidly in gelatine stab culture and do not liquefy the medium more rapidly than those cultures with attenuated virulence cultivated directly from feces.

In litmus glucose gelatine culture incubated anaërobically at 37° C for 48 hours, there is an abundant heavy, white, flocculent growth. The gelatine is completely liquefied. The litmus indicates an acid reaction, the medium having a bright red color. The odor is like butyric acid and also slightly putrefactive. The rods are large and plump and bear capsules. No spores are formed.

Serum gelatine stab cultures incubated aërobically at 60° F show about the same growth as glucose gelatine stab cultures incubated aërobically at 60° F, except that the serum is discolored green in the upper layers of the medium. These results would seem to confirm the statement made by Kamen[16] that the liquefaction of

gelatine depends largely upon the consistency of the medium and the temperature of incubation.

A starch-agar stroke and stab culture incubated 24 hours at 37° C contained abundant growth in the stab and liquid of condensation with the formation of many gas bubbles which riddled the medium. The gas formation in this medium is much more abundant than upon plain agar. The rods are large and plump, contain no granulose and no spores. The medium is made by adding 1 part of 2% corn starch solution to 3 parts of plain agar and sterilizing the medium for 15 minutes at 120° C.

Potato-agar stroke and stab cultures incubated aërobically for 24 hours at 37° C contain abundant growth with enormous formation of gas. The rods developing upon this medium contain granulose. Strains D265 (1), G264, H204, F225, cultivated upon this medium produce the same growth and the bacilli contain granulose.

A sugar-free bouillon and coagulated egg-white fermentation tube culture incubated for 24 hours at 37° C contained a moderate amount of growth. The closed arm of the tube contained 1 cm. of gas; the egg-white was only slightly decomposed. The tube had a slight odor of butyric acid. The bacilli were somewhat shorter and less plump than when grown in sugar-containing media and contain no spores. Longer incubation showed no further decomposition of the egg-white. Control cultures of the Bacillus of symptomatic anthrax and of the Bacillus of malignant oedema cultivated upon this medium digest the egg-white completely within 6 days, incubated at 37° C, and the tubes have a foul, putrefactive odor. The medium is prepared by adding a few pieces of coagulated egg-white, about 1 gram, to a fermentation tube containing sugar-free bouil-

lon and then sterilizing the tube at 120° C for 15 minutes. The bouillon is made sugar-free by the growth of a culture of Bacillus coli upon the beef juice from which it is made.

Coagulated egg-white and physiological salt solution cultures incubated anaërobically for 10 days at 37° C contain only slight growth. The medium is only slightly cloudy, the egg white is intact, is not softened nor discolored. The bacilli are few in number and usually contain granules which do not stain blue with Lugol's solution. No spores are observed. Cultures of the Bacillus of symptomatic anthrax and the Bacillus of malignant oedema cultivated upon the same medium as controls, do not decompose the egg, the growth is not very rich, but spores are formed. When 3% glucose is added to the medium the growth is somewhat more abundant and a little gas is formed. These do not confirm the results of Achalme[2] who obtained abundant growth with spore-formation in cultures upon this medium which had been sterilized at this temperature. The medium is prepared by adding 10 c.c. of physiological salt solution to 1 to 2 grams of coagulated egg-white in test-tubes and sterilizing the medium at 120° C for 15 minutes. There is no digestion if peptone solution is used instead of the salt solution and the growth is not more abundant.

Passini[28] described an egg-white medium which we have employed. It is prepared in the following manner: About 2 grams of coagulated egg-white are placed in a test-tube and heated in the autoclave at 144° C for 1 hour. The brown-colored, partly firm mass is then immersed in a few c.c. of sterile peptone solution or sterile bouillon. Cultures upon this medium incubated for 15 days at 37° C anaërobically completely dissolve the egg, the semi-

solid brown mass becoming a brown-colored liquid. If carbohydrate is added (3% glucose solution) to the medium, gas is formed and the egg is not dissolved. If the inoculation has been made with spore-material and no carbohydrate has been added to the medium, spores are formed, but if carbohydrate has been added no spores are formed.

No growth is obtained by inoculation of the bacilli into distilled water containing fibrin, after 10 days' incubation at 37° C anaërobically. Cultures of the Bacillus of symptomatic anthrax and the Bacillus of malignant oedema produce no growth upon this medium. The medium is prepared by adding a few pieces of blood fibrin, which has been washed free from corpuscles, to about 10 c.c. of distilled water and sterilizing the medium at 120° C for 15 minutes.

The bacillus does not digest meat in cultures incubated anaërobically for 10 days at 37° C. The meat is not digested nor discolored, the tube has a butyric acid odor and the growth is not very rich. The rods are somewhat longer and more slender than are ordinarily found. No spores are formed. The medium is prepared by adding about 1 gram of meat to 10 c.c. of distilled water in a test-tube and sterilizing the tube at 120° C for 15 minutes. The meat is not digested in cultures upon the medium which had been heated at 144° C for 1 hour.

Incubated Rabbit Test.- Welch[34] observed that Bacillus aërogenes capsulatus is capable of very rapid development in the blood vessels and tissues of an animal or in the human body after death and has described the so-called "incubated rabbit test" for his bacillus. This test gave the following results for strain D265 (1).

At 10:45 a. m. 12-16-'08 a gray rabbit was injected intravenously with about $\frac{3}{4}$ c.c. of a sugar blood bouillon fermentation tube culture of D265 (1). The growth was characteristic in the fermentation tube culture and the bacilli showed capsules when stained with the capsule stain. The inoculation was made into the marginal ear vein and 2 to 3 minutes were allowed for the culture to circulate thoroughly with the blood. The animal was then killed by a sharp blow upon the back of the head, placed in a battery jar and incubated at 37° C until 2:30 p. m., 12-16-'08 and then an autopsy was made.

Autopsy.- There is a very slight odor of putrefaction and butyric acid. Herter has described this odor as an odor of "saccharo-butyric fermentation". Bloody, frothy fluid oozes out of the mouth. The subcutaneous tissue over the abdomen and extremities crepitates with gas. The peritoneal cavity is tightly distended with gas which burns with a blue flame of hydrogen and gives the "hydrogen bark". There is bloody, frothy fluid in the inguinal and axillary regions. The heart muscle is firm. The heart contains bloody, frothy fluid. The liver is not discolored but is riddled throughout with gas. The intestines are inflated with gas which burns with a blue flame.

Microscopic Findings.- Coverglass smears from the heart blood, liver, fluid in the axillary region and subcutaneous tissue show the large, plump rods in pure culture. These rods bear capsules which are demonstrated by Hiss' capsule stain.

Cultures.- The bacillus was obtained in pure culture from the inguinal and axillary fluid, from the subcutaneous tissue over the abdomen and extremities, also from the heart blood and liver upon

blood agar anaërobic plates, in sugar blood bouillon fermentation tube, milk fermentation tube, and plain agar stab.

This test was applied to two other strains, F127 (1) and I137 (1) with similar results. In some cases where the rabbit was incubated 24 hours the odor became very strong and the decomposition greater. The muscles were almost completely decomposed.

c. Physical and bio-chemical characters.

Fermentation tubes containing sugar-free bouillon and	Dextrose 2%	Levulose 2%	Lactose 2%	Saccharose 2%	Maltose 2%
Gas production in percent..........	100	78	71	37	36
$\frac{H}{CO_2}$	$\frac{2}{1}$	$\frac{2}{1}$	$\frac{2}{1}$	$\frac{3}{1}$	$\frac{3}{1}$

NOTE.- In case of dextrose, levulose, lactose and maltose the formation takes place in 24 to 48 hours. Saccharose is slower; the formation of gas is complete in 4 days and in some cases no gas is formed at all.

Toleration for acids.- 2.92 c.c. of N-10 sodium hydroxide were required to neutralize the acids in 5 c.c. of a 48-hour old culture in 2% glucose bouillon grown anaërobically.

Sugar-free beef tea + 2% sugar (dextrose, levulose, lactose, saccharose, or maltose) + 10 to 12 drops sterile dog's, cat's or rabbit's blood. In fermentation tube cultures incubated 24 hours at 37° C the closed arm contains 100% gas, the blood is decomposed and tube has a strong odor of butyric acid. The rods grow large and bear capsules. No spores are formed.

Cultures grown in sugar-free bouillon 5 days show no test for indol.

d. Pathogenicity.- A series of inoculation experiments was made to test the pathogenicity of the bacillus. The following cultures were used for inoculation :

Culture I-D265 (1) original culture cultivated from feces.

Culture II-D265 (1) from the caseous tissue of a guinea pig injected intraperitoneally with culture I and dead with subcutaneous abscess.

Culture III-D265 (1) from heart of incubated rabbit inoculated with Culture I.

Culture IV-D265 (1) from the muscle of guinea pig dead after inoculation with Culture III.

Experiment I.- A guinea pig was injected intraperitoneally with about 1½ c.c. of a 48 hour dextrose bouillon culture of D265 (1) which had been recently plated and which gave characteristic colonies and showed capsules when grown in sugar blood beef tea fermentation tube. Injected at 3:50 p. m., 11-27-'08; animal acted sick immediately after inoculation. Nov. 28-'08, sat drawn up in the cage all day, ate and drank little. Nov. 30-'08, sat drawn up all day; there is a puffed place on the abdomen over the site of inoculation - a subcutaneous emphysema. Dec. 2-'08, general condition better; subcutaneous swelling almost disappeared. Dec. 4, 5, 6-'08, condition improved. Dec. 7-'08, under throat and over diaphragm are dark red sores with pus; skin is broken in places. Dec. 8-'08, general condition apparently the same; animal restless. Dec. 9-'08, guinea pig died at about 9:00 a. m. Death was slow and there was evidence of struggling. Autopsy at 10:45 a. m., 12-9-'08. No foul odor noticed; hair does not come off easily; a patch of skin about ¾ inches x 3 inches on the ab-

domen just above the site of inoculation is decomposed and may be lifted off with forceps; beneath the skin is caseous substance resembling caseated tuberculous tissue; it is creamy white and crumbles when penetrated with a platinum needle; there is a similar patch under the right jaw; the lymph gland seems to be enlarged and decomposed into a large caseous mass. Heart and lungs appear normal; liver is very dark red; intestines normal; the entire body tissue is darkened; no gas; muscles and subcutaneous tissue of the extremities appear normal.

Microscopic findings.- Smears from caseous tissue under jaw stained with Methylene blue show pus cells, no bacteria. Smears from heart blood show no bacteria.

Cultures.- Caseous tissue + sugar blood beef tea fermentation tube 24 hours at 37° C.— Closed arm filled with gas; blood decomposed; characteristic odor of "saccharo-butyric fermentation"; large plump non-motile rods are present which are capsulated. Caseous tissue + plain agar streak and stab 24 hours at 37° C, an abundant surface growth composed of a mixture of cocci and diplococci of different sizes and short rods; good growth in stab, with formation of gas bubbles, which is composed of a mixture of cocci and also displococci and short rods as seen upon the surface, also large plump non-motile rods. Heart blood + sugar blood fermentation tube 48 hours at 37° C, 4.4 cm. gas in closed arm, no odor of "saccharo-butyric fermentation"; growth is composed of a variety of organisms, cocci, large and small diplococci, short and some large rods in chains. These long rods do not bear capsules and appear narrower than the strain inoculated. A blood agar anaerobic plate inoculated with caseous tissue, incubated 3 days at 37° C

anaërobically shows typical deep oval and thin surface hemolytic colonies which are composed of the large plump non-motile rods without spores. Subcultures from these colonies show growth and morphology of rods typical for the strain originally inoculated into the guinea pig. A blood agar anaërobic plate inoculated from heart blood shows no rods of the type inoculated, only colonies composed of diplococci develop.

Experiment II.- A guinea pig was inoculated subcutaenously with about 1½ c.c. of a sugar blood bouillon fermentation tube culture of Culture I, original culture cultivated from the feces, at 11:40 a. m. 12-17-'08. Within a few hours after the inoculation the skin became puffed up with a large gas bubble over the site of inoculation and a watery fluid oozed out at the needle-pricked places in the skin. Within 24 hours the hair had fallen off over the site of inoculation and the skin looked white and cooked (due to acid in culture?), strong odor of butyric acid. Within a few days the gangrenous area spread and became firm and had a putrefactive odor; later it looked hard and yellow and caseous. This area covered the whole area over the diaphragm and upper part of the abdomen. Dec. 28-'08 this hard caseous slough had slipped off and left the clean red subcutaneous tissue which (12-29) shows signs of healing. Jan. 5-'09, wound appears to be healed. The animal recovered.

Experiment III. ¾ c.c. of a saccharose bouillon + sterile blood fermentation tube culture of culture I (the original culture cultivated from the feces), D265 (1), was injected into the breast muscle of a pigeon at 11:45 a. m., 1-26-'09. Within a few hours after inoculation the muscle was considerably darkened and swollen

with gas. Jan. 27-'09, pigeon died about 10:00 a. m. Autopsy at 10:45 a. m., 1-27-'09. No odor observed; feathers do not come off easily; skin looks green; the skin cuts tough and is oedematous, a gelatinous oedema, covered with a yellow mass, probably pus cells. This subcutaneous tissue has exactly the same appearance as in case of guinea pig inoculated with culture II D265 (1). This tissue is present all over the breast and back and extends a little into the wings and legs. The underlying muscle over the entire body is very red and around the site of inoculation is much swollen with gas and the muscle immediately surrounding the needle puncture is digested into a red jelly-like mass, to the bone. The breast muscle on the side not inoculated is fairly firm, also the muscles of the legs are firm. Gas is present in the subcutaneous tissue; the heart muscle is much reddened and contains a dark jelly clot; the muscle is firm; the liver is very dark red and softened; it does not contain gas.

Microscopic findings.- Coverglass smears from the breast muscle, subcutaneous tissue, legs, heart and liver show the rods typical for the culture inoculated; they appear to be present in pure culture and bear capsules. The rods are present in largest numbers upon the side of the breast muscle inoculated, fewer in the leg muscle and liver. The rods in smears from liver appear larger than those from other places.

Cultures.- The bacillus was obtained in pure culture from the breast muscle, leg muscle and heart blood.

Experiment IV.- A guinea pig was injected subcutaneously with 1½ c.c. of a sugar blood bouillon culture from the caseous tissue of guinea pig dead after inoculation with culture I (see

Experiment I) that is, culture II D265 (1) at 11:20 a. m., 12-17-'08. Within 24 hours after inoculation there was swelling over the site of inoculation due to gas. Animal sat drawn up in the cage and ate nothing. Dec. 18 and 19, subcutaneous tissue over site of inoculation hardened. Slight foul odor observed. Dec. 20, animal ate little and limped about the cage because of a large swelling around the left fore-leg. Dec. 21, 6:30 p. m. animal was found down in the cage in a dying condition. At 7:30 p. m. the animal was etherized and the autopsy made immediately. Skin over the abdomen is broken in places and can be torn easily; skin over the ribs tough; a large swelling around the left fore-leg; subcutaneous tissue of the neck and over the ribs reddened, but not digested; the swelling around the fore-leg is caused by a growth of caseous tissue which is yellowish-white and which resembles caseous tuberculous tissue; this material is found spreading under the skin of the abdomen and over the lower ribs. This tissue is rather tender and may be broken easily with forceps and resembles cooked or partially cooked meat in color; some of it seems "waxy" and has a pale yellow color. This pathological change causes a marked thickening of the abdominal wall increasing the subcutaneous tissue. Muscles of the fore and hind legs are not digested. Lungs are red, otherwise look normal; heart muscle is firm and is normal in color; heart blood when withdrawn is still fluid and rather bright red in color; diaphragm and intercostal muscles firm and intact; liver is normal except for a mass of caseous tissue lying on the ventral side directly below the subcutaneous lesion; this is a tissue change in the liver and resembles in appearance that described above for the subcutaneous

tissue. The intestines appear normal.

Microscopic findings.- Coverglass smears from the caseous tissue of the fore leg show capsulated rods which are few in number and other rods not capsulated (Hiss' Capsule Stain); smears from the heart blood show no bacteria; smears from liver about one or two rods per microscopic field.

Cultures.- The bacillus was obtained in pure culture from the heart blood and in mixed culture from the caseous tissue over abdomen, and from around the fore leg and from the liver. The contamination is due to other rods and to diplococci.

Experiment V.- A guinea pig was injected subcutaneously with about 1½ c.c. of culture III D265 (1), sugar blood bouillon fermentation tube culture from the heart blood of rabbit incubated after inoculation (intravenous) with culture I D265 (1), at 10:55 a. m., 12-17-'08. The guinea pig acted restless and sick immediately after inoculation; it was found dead at 8:00 a. m., 12-18-'08, less than 24 hours after inoculation, had not been dead long however, no signs of rigor mortis and body still warm. Autopsy at 10:30 a. m., 12-18-'08. No foul odor noticed; skin is puffed up with gas over the site of inoculation; no gas beneath the skin elsewhere; hair comes off easily and the skin is tough, but looks green; the entire subcutaneous tissue is very red and oedematous; the muscles of the fore legs, neck and intercostal muscles are partially digested and very soft, and pieces may be torn off easily with forceps; muscles of the hind legs are not digested; no fluid is observed. Lungs are bright red and mottled and look congested; heart muscle is firm, the jelly clot within it is dark; the diaphragm muscle is entire, not digested; liver is soft, not discolor-

ed; contains no gas bubbles; stomach and intestines normal.

Microscopic findings.- Coverglass smears from the subcutaneous tissue show the bacillus in pure culture; smears from the neck muscle stained by Hiss' capsule method show the capsulated rods and pieces of partially digested muscle fibers (the rods are found singly or in pairs and are much shorter than those ordinarily obtained from sugar blood bouillon fermentation tube cultures or from the tissues of rabbits incubated after inoculation with the bacillus). Smears from the heart blood show only a few capsulated bacilli; smears from liver show no bacteria.

Cultures.- The bacillus was obtained in pure culture from the heart blood and from the muscles.

Experiment VI.- About $\frac{3}{4}$ c.c. of a sugar blood bouillon fermentation tube culture of culture IV D265 (1) (from the muscle of guinea pig dead after inoculation with culture III) was inoculated into the breast muscle of a pigeon at 9:45 a. m., 12-22-'08.

About 2 hours after inoculation the muscle is much swelled ; skin is drawn tight over the site of inoculation. 4:30 p. m., 12-22-'08 pigeon found dead in the cage; body still warm. Autopsy at 5:30 p. m., 12-22-'08. Subcutaneous tissue is crepitant with gas; breast muscle on the side which was inoculated is red and much swollen. In the inguinal region on the inoculated side there is swelling and reddening of the skin; slight butyric odor is noticed; when skin is removed, the breast muscle on the side which was inoculated is seen to be decomposed badly; there is partial digestion and softening and the whole muscle crepitates with gas bubbles; it is very red and is swelled double the size of the opposite side; at the point of inoculation the muscle is

completely digested into a red, clotted mass to the bone; the muscle on the side of the breast not inoculated is red but firm and contains no gas bubbles ; the muscles of the legs look normal and are not digested; bloody fluid in inguinal region; lungs are red; heart muscle is softened; liver appears normal; gizzard and intestines appear normal; much gas thruout the subcutaneous tissue.

Microscopic findings.- Coverglass smears from heart blood, liver and bloody fluid in inguinal region show no bacteria; smears from breast muscle on the side inoculated and subcutaneous tissue over the site of inoculation show capsulated rods in large numbers,pure (Hiss' capsule method); smears from the breast muscle on the opposite side show capsulated rods present in small numbers in pure culture.

Cultures.- The bacillus was cultivated in pure culture from heart blood, liver, breast muscle and subcutaneous tissue. Sections from the breast muscle,heart and liver were stained in haematoxylin and eosin and by the Gram-Weigert stain. Sections from the breast muscle show the muscle fibers broken up both longitudinally and cross-wise and the bacteria are found around the muscle bundles and in the blood-vessels. There is no small-celled infiltration, no reaction on the part of the tissue.

Summary of the Experiments upon Pathogenicity

Experiments I and II show that the strain isolated from the feces is pathogenic for guinea pigs. An intraperitoneal injection, in the case of Experiment I causing death of the animal, while a guinea pig recovered from an equal dose when injected subcutaneously. In both cases there was considerable reaction on the part of the tissue and secondary invasion of other bacteria.

Experiments II, IV and V were made at the same time for the purpose of determining if the virulence of the strain is increased by passage through the animal body. It was found that the virulence is greatly increased. In Experiment II, the guinea pig recovered from an inoculation of a culture which had not been passed through another animal. In Experiment IV, the guinea pig having been inoculated with an equal amount of a culture, incubated under like conditions, but which had been obtained from the caseous tissue of the guinea pig in Experiment I, died in 3 days, showing an increased virulence by a single passage through an animal. In Experiment V, the guinea pig died in less than 24 hours, after an injection of a culture from the heart blood of an incubated rabbit. In this experiment the same amount of a culture incubated under the same conditions as in Experiments II and IV was inoculated. This experiment (Exp. V) shows that the virulence is much increased by cultivation in the dead body. In Experiments IV and V there was rapid liquefaction of the tissues with abundant gas formation and little reaction on the part of the tissue. In Experiments III and VI the pathogenicity to pigeons was tested. In Ex-Experiment III a pigeon inoculated with the culture which had not been passed through any animal died in less than 24 hours with little reaction on the part of the body tissue. This shows (Exp. I and III) that pigeons are more susceptible to the poisons of the bacillus than guinea pigs. In Experiment VI, a pigeon was inoculated with a culture which had been passed through two guinea pigs and died in less than 7 hours, showing a higher degree of virulence.

The experiments show that the culture isolated from the feces is pathogenic when inoculated into pigeons and guinea pigs and

that the virulence is increased by passage through an animal (Exp. IV) and by incubation in the dead body of a rabbit so that it is as virulent as the strains which Welch obtained from cases of the infection in man (Exp. VI).

Summary and Conclusions

1. Strains of Bacillus aërogenes capsulatus may be isolated from the intestine of man in a normal state of health, regularly, in dilutions as high as 1 to 1000 and 1 to 5000.

2. The bacillus is best obtained from the pasteurized spore-material, which is inoculated into special anaërobic media.

3. The morphology and the cultural properties of the bacillus correspond well with those described for the Bacillus aërogenes capsulatus by those who have investigated it.

4. The strains isolated, although they were originally obtained from spore-material, form spores with difficulty upon artificial media.

5. These strains, which are under ordinary conditions of cultivation, asporogenic, may be transformed into the sporogenic form by repeated transplantations of spore-material from a preceding transplant upon Loeffler's blood serum or plain blood serum or upon egg-white medium which has been heated at 144° C for 1 hour.

6. The cultural tests show that the fermentative activity of the bacillus, as isolated from the intestine, is more energetic than the putrefactive activity.

7. The strains studied do not digest the egg-white in a medium which has been sterilized at 120° C for 15 minutes, but dissolve the egg-white in a medium which has been heated at 144° C

for 1 hour.

8. The strains studied liquefy Loeffler's blood serum more slowly than cultures of the Bacillus of symptomatic anthrax and the Bacillus of malignant oedema incubated under similar conditions.

9. In cultures grown upon potato and potato-agar some of the rods contain granules which stain blue with Lugol's solution.

10. The strain of the bacillus studied in detail is pathogenic for guinea pigs and pigeons, the latter being very susceptible. The cultures are not highly virulent.

11. By passage through an animal, cultures become highly virulent and when inoculated into guinea pigs and pigeons produce a rapid digestion of the tissues, with abundant gas formation followed by death in less than 24 hours.

It is evident that no tests have been made with the object of approaching the exact conditions which obtain in the intestine. Since the bacillus has both the power of fermenting carbohydrates, and of splitting proteins it is not unlikely that both of these activities may go on in the intestine; whether or not both may go on at the same time is a question. An important fact shown by the cultural tests is that whenever there is fermentable carbohydrate present in the medium in any considerable amount, the proteins in the medium are not split up into their putrefactive products. This result would tend to show that fermentation and putrefaction by this bacillus do not go on at the same time under any conditions and which one does occur depends upon the nature of the culture medium. Bienstock[3] was the first to point out that the growth of a putrefactive anaërobic bacillus is inhibited by the

bacilli of the Coli group. He found that his Bacillus putrificus did not develop well in mixed culture with Bacillus coli. Passini[29] and others have worked upon this property of the colon bacillus and have tested it with other anaërobic bacilli, among them, Bacillus aërogenes capsulatus. The question as to the exact nature of the activity is unsettled. This inhibitory action may be strikingly and simply demonstrated by inoculating two litmus milk fermentation tubes, one with a mixed fecal suspension which contains the colon bacilli in considerable numbers and also spores of anaërobes and inoculating the other with the heated spore material, as described above. In the case of the former, after 24 hours incubation at 37° C there will be found some gas in the closed arm of the tube, 1 to 5 cm., the milk will be coagulated and the litmus partially reduced. The Gram-stained sediment shows few, if any, large plump positive rods, while rods of the Coli type and other bacteria are present in large numbers. The fermentation tube inoculated with the heated material shows the "stormy" fermentation characteristic of Bacillus aërogenes capsulatus. All the authors agree that this inhibitory action is manifested in the intestine. The efficiency of this inhibitory action depends upon many factors, such as, diet, condition of the different parts of the alimentary canal, and others. Considering these factors, it is difficult to draw conclusions from results obtained in the test tube and one is in no wise justified in applying them to the bacterial growth as it occurs in the intestine.

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